liver tissue was ground in the Waring blender indicated that the mitochondrion was not merely a "sac containing a solution of soluble oxidative enzymes but . . . an organized structure with an insoluble matrix in which are embedded the individual catalytically active proteins making up the complex enzymatic machinery of oxidation and phosphorylation" (Lehninger, 1951, p. 12). Further influenced by the difficulty of accounting for the rates of oxidative phosphorylation by diffusion of intermediates, Lehninger advanced a bold proposal: "It would appear that these carrier proteins must be fixed in space so that hemes are juxtaposed, increasing the probability of fruitful collision, or that special mechanisms must exist to allow passage of electrons through the protein moieties" (p. 12).[7] Such a proposal departed radically from the traditional biochemical focus on soluble enzymes and helped set the stage for linking biochemical processes with cell structures.

David Green was another biochemist who responded to the difficulty of isolating oxidative enzymes by advancing the idea that the enzymes involved in oxidative phosphorylation constitute an organized system. Green had established his reputation through empirical work purifying and characterizing enzymes involved in cellular respiration while working with Malcolm Dixon at Cambridge in the 1930s (Green, 1936a; Green, 1936b; Green & Brosteaux, 1936; Green, Dewan, & Leloir, 1937; Green & Dixon, 1934) and by the publication of his 1940 book, *Mechanisms of Biological Oxidations*, which provided many in North America with their primary introduction to enzyme chemistry.[8] In 1948 he advanced the immediately controversial claim that the

[7] The next step in Lehninger's program was to determine where along the electron transport chain ATP synthesis occurs. He attempted to study the reactions starting with ascorbate (ascorbic acid, a nonenzymatic reductant of cytochrome *c*), and confronted the same difficulty – only when preparations were pretreated was the electron transport accompanied by phosophorylation of ADP. But with pretreatment, Lehninger was able to establish that one phosphorylating event occurred between cytochrome *c* and oxygen (see Lehninger, 1954). This implied that the other two phosphorylating events must occur earlier in the chain. Slater (1950) had previously found evidence for at least one phosphorylation earlier in the chain by using α-ketoglutarate (α-ketoglutaric acid) as the hydrogen donor and ferricytochrome *c* as the acceptor, and the question of exactly where in the electron transport chain the coupling with phosphorylation occurred remained a focus of inquiry throughout the 1950s. A major technique for approaching the problem was developed by Chance and Williams (1956). They used spectrography to determine the oxidation state of intermediates and, employing various inhibitors to impede the overall reaction and then adding ADP, they were able to identify zones in which each phosphorylation occurred.

[8] Green returned to the United States at the beginning of World War II and after a short period at Harvard, was appointed in 1941 assistant professor of biochemistry in the Department of Medicine at the Columbia College of Physicians and Surgeons in New York City. While he was in the midst of formulating the cyclophorase concept, he was approached by the University of Wisconsin, already a top institution in biochemistry, to become a founding member of the Institute for Enzyme Research at the University of Wisconsin. Green recruited an extremely